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OBSERVATIONS ON MICROFOULING APPLICABLE TO OTEC SYSTEMS

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ABSTRACT

Solid surfaces exposed to seawater become populated with bacteria in as little as four hours. Subsequent colonization by a variety of microorganisms produces a complex microfouling layer including their extracellular metabolites and cellular breakdown products, water-borne detrital material, and metal corrosion products (on metal surfaces). The presence of such a primary film on a heat exchange surface may well hinder heat transfer and may be critical to an OTEC system already operating at a low theoretical Carnot efficiency. Furthermore, the metabolic activity within this microcosm may enhance corrosion processes.

The succession of periphytic microorganisms was observed for a variety of surfaces, including glass, stainless steel, brass and copper-nickel alloys, submerged in natural seawater. The nature of the periphytic community was influenced more by the composition of the substratum than by the nature of the background planktonic microbiota.

INTRODUCTION

The deployment of Ocean Thermal Energy Conversion (OTEC) facilities for tapping the naturally abundant oceanic thermal energy gradients is attractive, particularly at a time when energy demands are soaring and resources are dwindling. The small temperature differential between condenser and evaporator waters endows OTEC facilities with low theoretical Carnot efficiency, thus requiring that the caloric turnover through the plant be larger than in conventional counterparts for an equivalent of usable energy. Therefore, a large volume of water will have to pass through the heat exchange system, and the surface area to volume ratio of this system should be high as should its thermal conductivity.

It has long been recognized that surfaces subjected to seawater become fouled with microorganisms as well as detrital material (Zobel and Allen, 1933; and many others through the years). Laity (Haderlie, 1977) and Stupian (1976) calculated that primary fouling films 25 microns and 250 microns thick would cause, respectively, a 10% and a 50% reduction in the heat transfer coefficient of heat exchanger tubes. These considerations draw attention to a limiting factor in an OTEC plant operation, namely the microfouling of heat exchanger tubes and the consequent deterioration of their heat transfer properties. Haderlie (1977) reviewed the subject of organic and microbiological films in the marine environment and their significance for OTEC heat exchange surfaces. In addition to these, Haderlie points out another potential problem associated with primary foulings -- microbial corrosion.

The implication of bacterial activities in corrosion processes was recognized as early as 1891 when Garrett postulated that corrosion of lead could be due to ammonia, nitrites and nitrates produced by bacterial action. Since then, an increasing volume of information has been gathered implicating microorganisms in corrosion processes in various environments. Discussion of the various mechanisms that may be operating in microbiological corrosion is outside the scope of this paper and may be found in publications of, among others, Costello (1969) and Iverson (1972). It is interesting and somewhat alarming that in spite of the available information on the subject, microbiological activity has traditionally been neglected as an important contributing factor in the marine associated corrosion. The naiveté of some scientists who consider seawater a simple solution of sodium chloride contaminated with a few additional salts was pointed out by Compton (1970), who called attention to the so-called "biological factors" which are so often mentioned with regard to the uniqueness of seawater. Since OTEC heat exchange surfaces would not be subjected to lethal temperatures (as in other power plants), the presence of a viable microbiological community on these surfaces should be expected. This community would probably manifest enhanced metabolic activities (Hendricks, 1974), and would therefore potentially be more destructive to metals than has thus far been envisioned.

The purpose of this paper is to report our observations on microfouling of glass, stainless steel, brass, and copper-nickel alloys exposed to a tropical marine environment. These observations will be discussed in the context of OTEC program objectives.

MATERIALS AND METHODS

Metal and glass samples were exposed in a fiberglass tank containing approximately 15 m^3 of seawater. Water depth was maintained at 1.2 m over 35 cm of locally derived carbonate sediments. The exposure tank is located at Bear Cut, a tidal pass between Biscayne Bay and the ocean, and receives water alternately from the ocean and the Bay, depending on the phase of the semidiurnal tides. A once-through flow of seawater was continuously maintained with a turnover time of approximately 18 hours. The tank was under a natural light cycle, and its environment closely resembled that of adjacent Biscayne Bay.

SPECIMEN PREPARATION AND HANDLING

Coupons of stainless steel 304 (2.7 cm X 2.7 cm X 0.5 cm), 60/40 copper/zinc brass (2.5 cm X 2.5 cm X 0.6 cm), copper-nickel 90/10 and 70/30 alloys (1.4 cm X 1.4 cm X 0.3 cm), and glass microscope slides were prepared, weighed, and placed in seawater as described by Gerchakov et al. (1976). Precautions were taken in placing and harvesting specimens to prevent them from contacting the air/water interface, thus eliminating contamination by bacterioneuston. Such contamination could result in an increase of up to three orders of magnitude in the periphytic bacterial population (DiSalvo, 1973). Replicate samples were allotted for bacterial, mycological, and scanning electron microscopic studies. An additional specimen was taken of each metal for weight-loss determination, a measurement which was repeated on specimens previously used in microbial studies.

SCANNING ELECTRON MICROSCOPY

Sample coupons were fixed in 4% glutaraldehyde in seawater immediately after their retrieval, and either critical point dried or air dried. Air dried samples were washed in distilled water, dehydrated in acetone, immersed in xylene and air dried. For critical point drying, samples were washed in distilled water, dehydrated in ethanol and critical point dried in Freon 13 using Freon TF as an intermediate fluid. Glass and metal samples were then coated with Au-Pd in a Denton vacuum evaporator and examined in an AMR-900 scanning electron microscope operated at 21 KV accelerating voltage.

MICROBIOLOGICAL ANALYSES

The procedure used for the study of bacterial, filamentous fungal, and yeast populations from glass, stainless steel, and brass specimens, were those described by Gerchakov et al. (1976). Some modifications were made in handling the Cu/Ni coupons and are described below.

For the culture of filamentous fungi and yeasts, the coupons were incubated on the surface of selective mycological medium for 48 hours and then inverted on the medium and incubated for an additional 48 hours; thus both sides of the specimens were cultured for resident periphytic eumycetes. After this 96-hour incubation period, the coupons were totally immersed in an enriched broth made selective for fungi by the addition of gentamicin and chloramphenicol (both at 40 mg/l) to inhibit bacterial growth.

It was found that the carbon-steel blades used for scraping specimens significantly marred the soft copper-nickel coupons. Consequently, the removal of the microfouling film for bacterial work was accomplished by means of a Teflon wedge. This technique proved to be more temperate and equally effective, as evaluated by scanning electron microscopy.

Bacterial Enumeration.

Scrapings were plated in duplicate on modified Difco Marine Agar 2216, MMA, (Gerchakov et al. 1976), and duplicate copper-nickel samples were prepared for examination by fluorescent microscopy by glutaraldehyde fixation and staining with acridine orange (Basic Orange 14, Matheson, Coleman and Bell; Norwood, Ohio). This direct counting procedure was done with a Leitz Ortholux microscope employing a Ploem illuminator system consisting of an HPO-200 lamp, heat filter, BG-38 exciter filter, one or two KP 490 exciter filters, TK 510 beam splitter, and a K510 barrier filter.

Plating.

Duplicate aliquots of scraping dilutions were filtered with suction through a Millipore filter (0.45 micron pore size, 47 mm diameter) on a Millipore Hydrosol Manifold filter apparatus. Each filter was placed on a MMA plate. Plates were incubated at room temperature up to 14 days. Colony counts were made at 2, 7, and 14 days and up to 20 colonies from a given quadrant or quadrants were sub-cultured for physiological and taxonomic purposes.

Direct Count.

Copper-nickel coupons were rinsed in sterile seawater, fixed in 4% glutaraldehyde for 20 minutes, rinsed several times in distilled water and stained 15 minutes in a 0.01% acridine orange solution using a modification of the Trolldenier (1973) method. Samples were air dried and observed at 950X (oil immersion). Orange fluorescing bacteria-like cells were counted in thirty fields per sample side.

An alternative method was used for direct counting when extensive corrosion of the copper-nickel surfaces made the above technique difficult. This method involved the use of Nucleopore filters which were prestained with irgalan black (Chemical Index, acid black 107; Hobbie et al. , 1977). Metal scrapings were filtered through the prestained filters (0.2 micron pore size, 47 mm diameter), fixed with glutaraldehyde, stained with acridine orange and observed by fluorescent microscopy.

Isolation and Storage of Bacterial Cultures.

Pure cultures were isolated by successive streaking on Marine Agar 2216 (Difco). These cultures were also microscopically examined to ensure their purity. Stock cultures were maintained in a lyophilized form, and at $4^{\circ}C$ in Cytophaga Seawater Agar (Anacker and Ordal, 1955) prepared using aged Gulf Stream water as a base. All isolates were also subcultured at 4-week intervals on Marine Agar 2216 slants.

WEIGHT-LOSS DETERMINATION

The procedure utilized to clean the metal coupons after exposure to seawater was standardized throughout this study so that internally consistent weight-loss data was obtained. In all cases, surfaces were examined under a dissecting microscope (100X) after cleaning to ensure that the surfaces were clean and to determine the extent of pitting corrosion. Briefly, the specimens were: (1) rinsed with distilled water (D.W.); (2) scrubbed with a nylon-bristle brush with a mild detergent solution; (3) placed in an ultrasonic bath for one minute with the detergent solution; (4) brushed; (5) rinsed with D.W.; (6) treated in an ultrasonic bath with D.W. for one minute; (7) rinsed with D.W.; (8)

rinsed with D.W. with brushing; (9) rinsed with D.W.; (10) rinsed three successive times with methanol; and (11) blotted dry.

RESULTS AND DISCUSSION

SCANNING ELECTRON MICROSCOPY

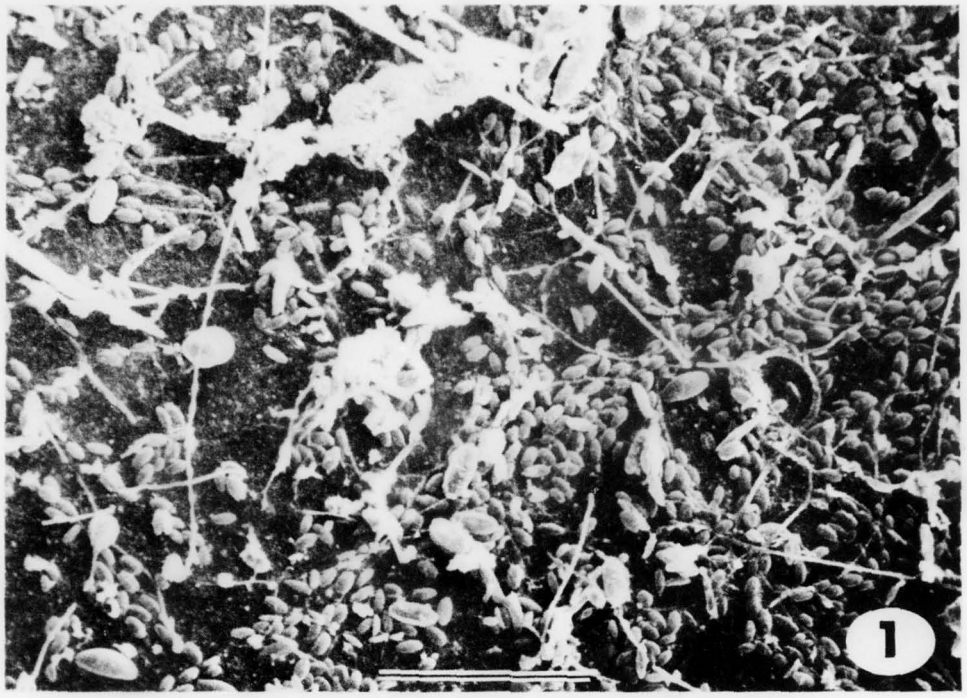
The development of biofouling layers was observed by scanning electron microscopy for a variety of substrates including glass, stainless steel, brass, and copper-nickel alloys 70/30 and 90/10. Although exposed under near-identical conditions each substrate influenced the succession of fouling microorganisms, especially during the early stages of exposure.

Glass biological slides, stainless steel and brass were exposed for four weeks during April and May, 1976. Microfouling of the glass and stainless steel closely followed the periphyte succession observed on several earlier runs of these materials (Gerchakov et al. 1976), and is summarized below.

Bacteria and fungi were the first organisms to colonize glass and stainless steel surfaces. After a few days, choanoflagellates (Figures 3 and 4 in Gerchakov et al., 1976) became numerous, and with the bacteria and fungi, remained the dominant periphytes through the second week of exposure. After two weeks, choanoflagellates and fungi decreased in abundance, while the number of bacteria continued to increase. Diatoms, filamentous algae, and ciliated protozoa appeared in significant numbers during the third week. Numerous species of attached and motile diatoms colonized the glass and steel surfaces and eventually (at about 4 weeks exposure) diatoms became the dominant periphytes. At about five weeks exposure, a two-tier fouling layer was

observed. The first-tier fouling organisms in direct contact with the substrate were mainly bacteria, fungi, and attached diatoms. Second-tier organisms were mainly large motile diatoms, peritrichous ciliates, filamentous algae, and numerous other organisms in lesser abundance. At that time, the fouling layer was visible to the naked eye and macroscopic organisms (worms, sponges, etc.) began to appear. A two-tier fouling layer characteristic of glass and stainless steel exposed for several weeks is illustrated in Figures 1 and 2.

Brass coupons exposed at the same time did not exhibit the succession of fouling microorganisms observed on either the stainless steel or glass control substrates. No microorganisms were observed on brass after four hours exposure, although isolated rod-shaped bacteria were observed on the glass control. Bacteria appeared on brass after one day exposure, along with minute flagellates less than 5 microns in diameter. Small colonies of 10 to 30 cells of rod-shaped bacteria were present at two days exposure; by six days exposure, colonies consisting of several hundred cells each were common. The flagellates increased in abundance during the second and third weeks of exposure. Choanoflagellates appeared on the brass at about 5 days exposure, rapidly increased in abundance during the second week, and became rare after 14 days exposure. Two species of choanoflagellates were observed, identical to those which colonized glass and stainless steel. Diatoms and filamentous algae were observed only rarely on brass exposed for more than two weeks, and these organisms remained numerically insignificant. The brass coupons were exposed for a maximum of 4 weeks during this initial experiment and at the end of that time period the fouling layer consisted of bacteria and their organic secretions, abundant small flagellates, and occasional diatoms.



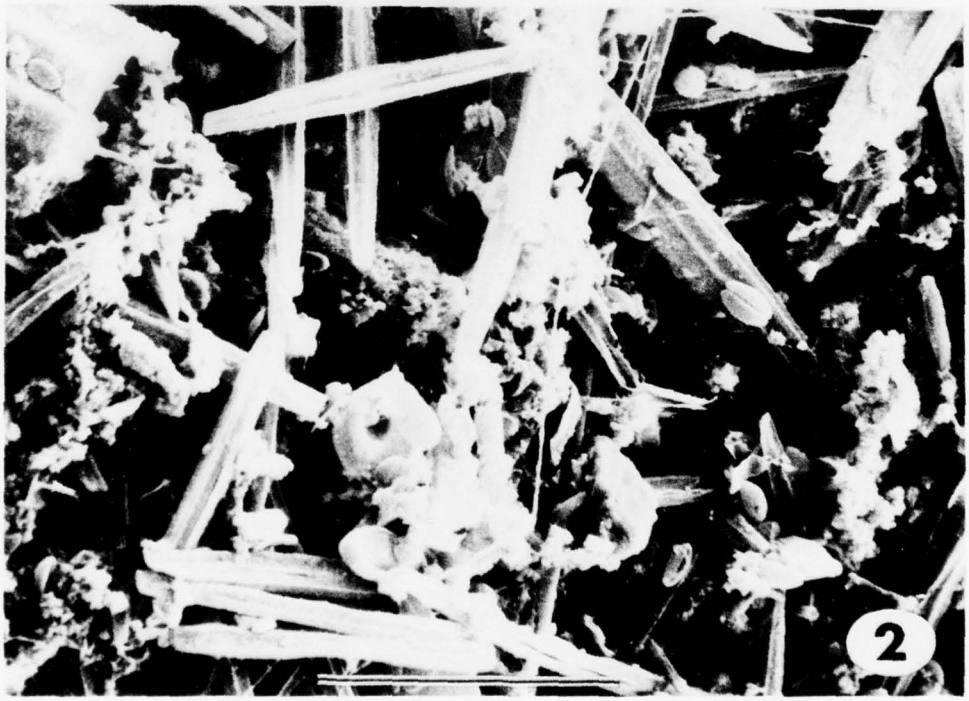


Figure 1. Microfouling typical of glass and stainless steel exposed for three weeks. Microorganisms attached to the substrate are mainly small diatoms, bacteria and fungi. Bar scale = 0.1 mm

Figure 2. Exposure of stainless steel for five weeks produces a two-tier fouling layer. Large motile diatoms, filamentous algae, bacteria, fungi, ciliates and other microorganisms overlie the first-tier organisms, and are not in direct contact with the substrate. Bar scale = 0.1 mm

Figure 3 illustrates rod-shaped bacteria on brass after 6 days exposure; continued exposure for more than two weeks showed bacteria associated with an organic "slime" assumed to be secreted by the cells (Figure 4). As seen in the micrograph, the bacteria were living within the slime at the time the brass coupon was prepared for scanning electron microscopy.

Most of the fouling microorganisms on the brass coupons were not in contact with the original polished surface but rather were attached to a sheath-like oxidized layer which developed during the first week of exposure to seawater. The oxide layer was composed of acicular crystallites approximately 0.5 micron in length, and was relatively uniform in thickness (Figures 3 and 4).

Copper-nickel 70/30 and 90/10 alloys and glass slides were exposed for three months during the summer of 1977. The glass substrate fouled as described earlier and the observed succession of microorganisms will not be repeated here.

Similar successions of microorganisms were observed by scanning electron microscopy on both copper-nickel alloys. Small flagellates (less than 5 microns diameter) were present on coupons examined after four hours exposure; bacteria were observed but were rare at that time. After one day exposure, scattered rod-shaped bacteria were present and flagellates had increased in abundance. At six days exposure a substantial increase in the bacterial population had occurred resulting in the formation of colonies. The most dramatic increase in abundance of bacteria occurred during the second week. During that time interval, the copper-nickel surfaces were characterized by irregular strands of rod-shaped bacteria and slime (Figure 5). Samples harvested during the third week showed that although bacteria were still abundant, the cells were more likely to occur as a single layer rather than as a layer several cells thick. Diatoms also appeared on the coupons during the third week, but were numerically unimportant. The

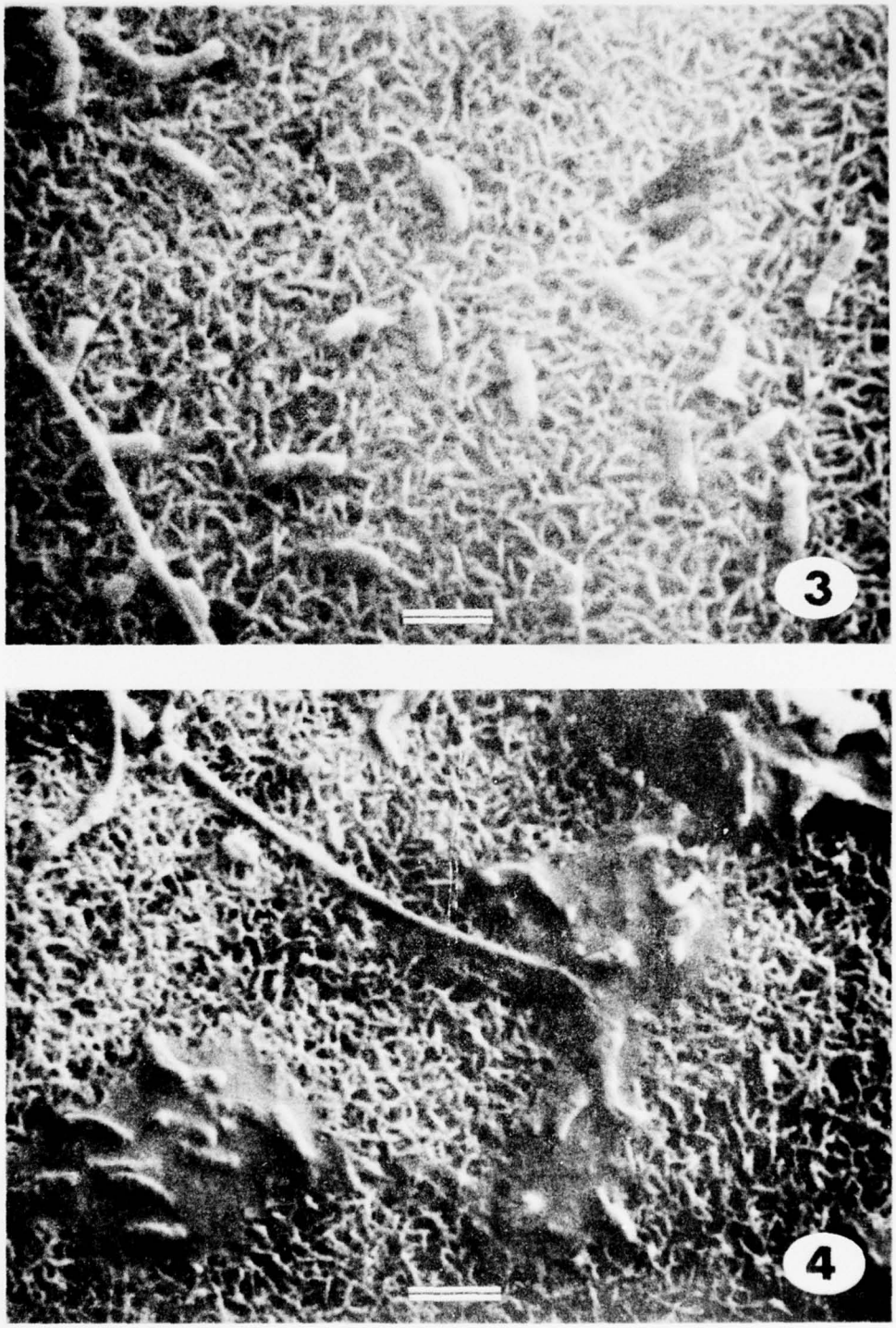


Figure 3. Brass. Dividing bacteria from colonies on "oxide" layer which appears during the first week of exposure. Six days exposure. Bar scale = 1 m

Figure 4. Brass. Continued exposure reveals bacteria associated with organic secretions. Sixteen days exposure. Bar scale = 1 m

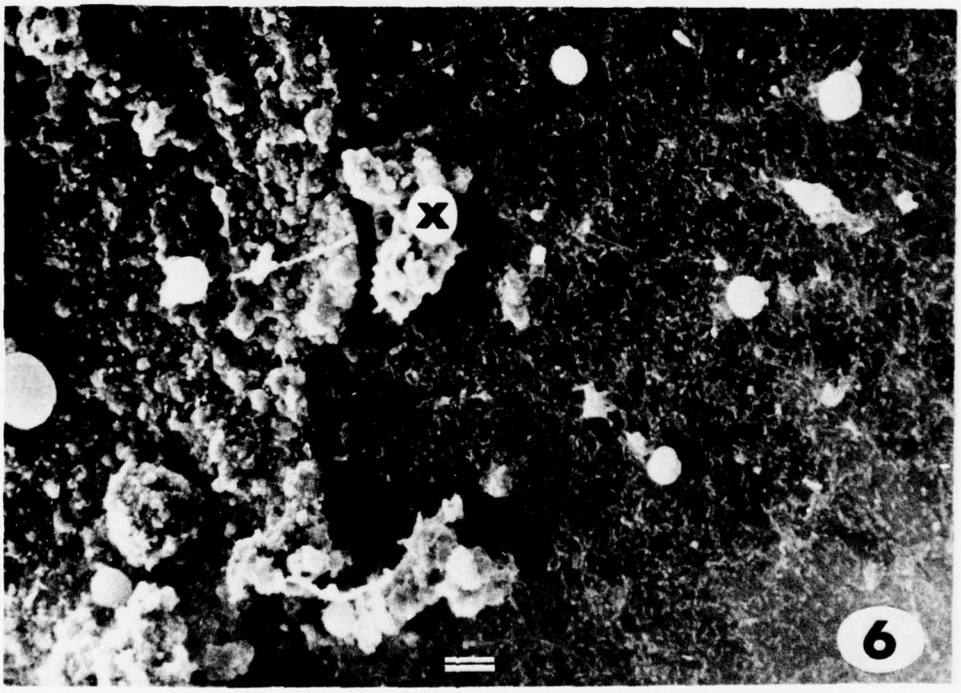


Figure 5. Copper-nickel 70/30 alloy. Bacteria and "slime" became abundant on copper-nickel surfaces during the second week of exposure. Bar scale = 10 m

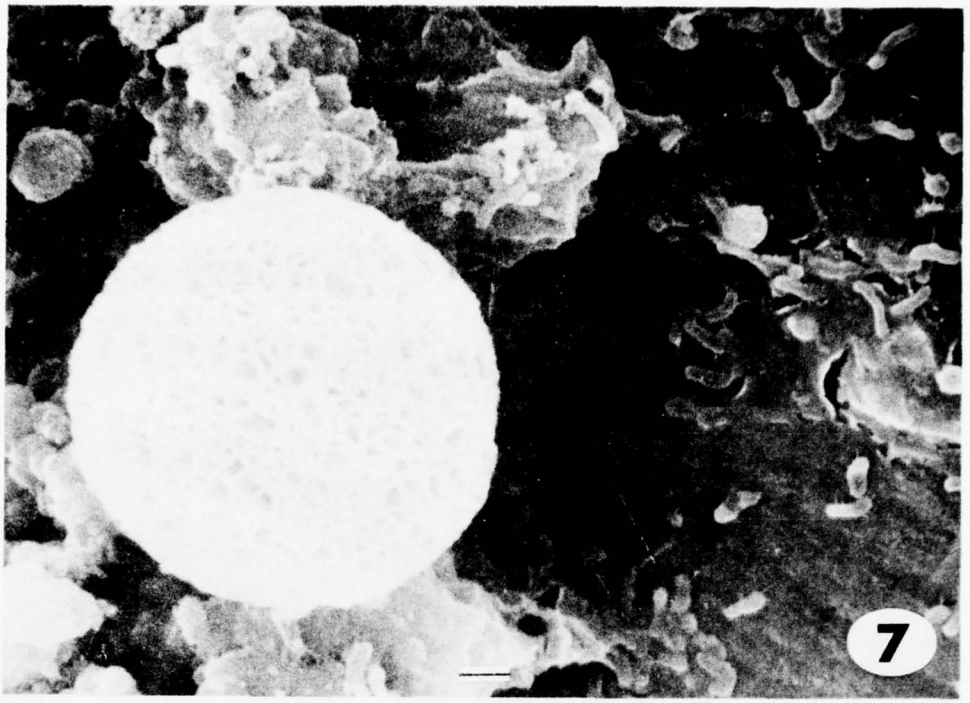
Figure 6. Copper-nickel 90/10 alloy exposed for more than two weeks produces a fouling layer composed of microorganisms and inorganic crusts (left side of micrograph). Spherical crystalline deposits (X) are also present. Bacteria are concentrated in crust-free areas (right side of micrograph). Bar scale = 10 m

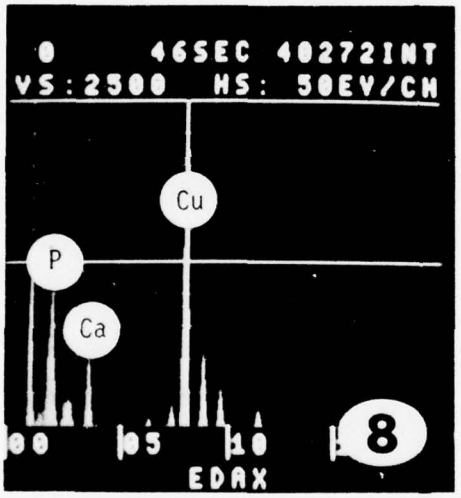
fourth week of exposure was marked by a rapid increase in diversity. Besides rod-shaped bacteria and flagellates, coupons examined after 27 days exposure were characterized by a fouling layer consisting of peritrichous ciliates (Zoothamnion sp.), several species of diatoms, colonies of coccoid bacteria, and fungal hyphae.

Although the successions of microorganisms observed on copper-nickel 90/10 and 70/30 alloys were basically similar, the physical characteristics of the fouling layers were substantially different on the two alloys. The 90/10 alloy developed an inorganic crust which appeared green in reflected light. The scanning electron microscope showed the crust to be discontinuous and of variable thickness (Figure 6). Flaking was apparent after six days exposure. Microorganisms were concentrated in areas of the 90/10 alloy coupons where crust development was minimal (Figures 6 and 7).

Compared to the copper-nickel 90/10 alloy, inorganic surface deposits derived from the base metal developed more uniformly and at a slower rate on the 70/30 alloy. Scattered crystalline deposits were observed on the surfaces of those coupons but they did not form a continuous crust during the length of the exposure period. The fouling layer on the copper-nickel 70/30 alloy harbored more fouling organisms than did the 90/10 alloy, perhaps because more crust-free surface was available for colonization.

Energy dispersive X-ray microanalysis of the inorganic crust developed on copper-nickel 90/10 alloy showed copper to be present in highest concentration, along with minor amounts of other elements (Figure 9). Selective analysis of spherical crystalline deposits observed on all the copper based alloys indicated that these characteristic deposits were complex salts composed mainly of the elements Cu/Ca/P (Figure 8). The analytical technique used is insensitive to





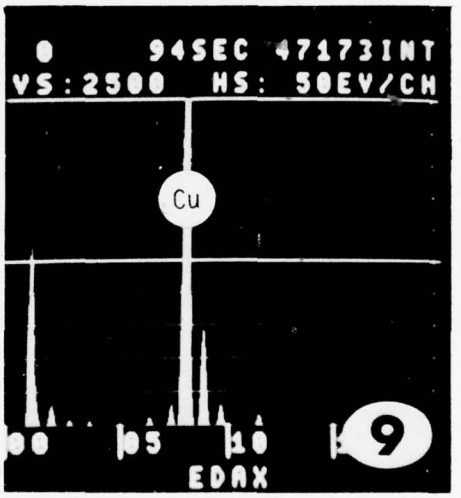


Figure 7. Detail of Figure 6. Spherical crystalline deposits (X on Figure 8) were observed on all copper based alloys. X-ray microanalysis of these deposits on 90/10 alloy indicates an elemental composition mainly of Cu/Ca/P. Bar scale = 1 m

Figure 8. Elemental composition of the spherical deposit shown in Figures 6 and 7. Peaks not labeled are derived from the Au-Pd conductive coating applied to SEM samples.

Figure 9. Elemental composition of the crust shown in Figures 6 and 7 indicates copper as the primary elemental constituent (see text for explanation).

carbon; however, the crystalline salts may well contain carbonate, especially if they have nucleated at cathodic microcorrosion sites.

In summary, development of microfouling layers on a variety of substrates as characterized by scanning electron microscopy appeared to be greatly influenced by the nature of the substrate. Of the materials examined so far, glass and stainless steel exhibited the maximum microbial diversity and abundance, and eventually produced two-tier fouling layers. Copper based alloys (brass, copper-nickel 90/10 and 70/30 alloys) showed less diversity and abundance, and produced biofouling layers in association with inorganic oxide layers and complex crusts.

MICROBIOLOGICAL ANALYSES

The enumeration of bacteria associated with microfouling films is by no means a simple task because no one method is capable of yielding accurate and reproducible numbers under all conditions. Our studies using Modified Marine Agar in conjunction with Millipore membrane filters were designed to consistently enumerate and isolate a portion of the heterotrophic bacterial populations.

The number of heterotrophic bacteria colonizing the surfaces of stainless steel, brass and copper-nickel were compared with those colonizing control glass surfaces.

Although the initial attachment of bacteria to the glass surfaces was most rapid, the subsequent attachment and/or growth on stainless steel was even greater than on glass (Figure 10). At 40 days, approximately 1.5×10^3 colony-forming units (CFU) were isolated per square centimeter of stainless steel surface while only 6×10^2 were cultured from glass.

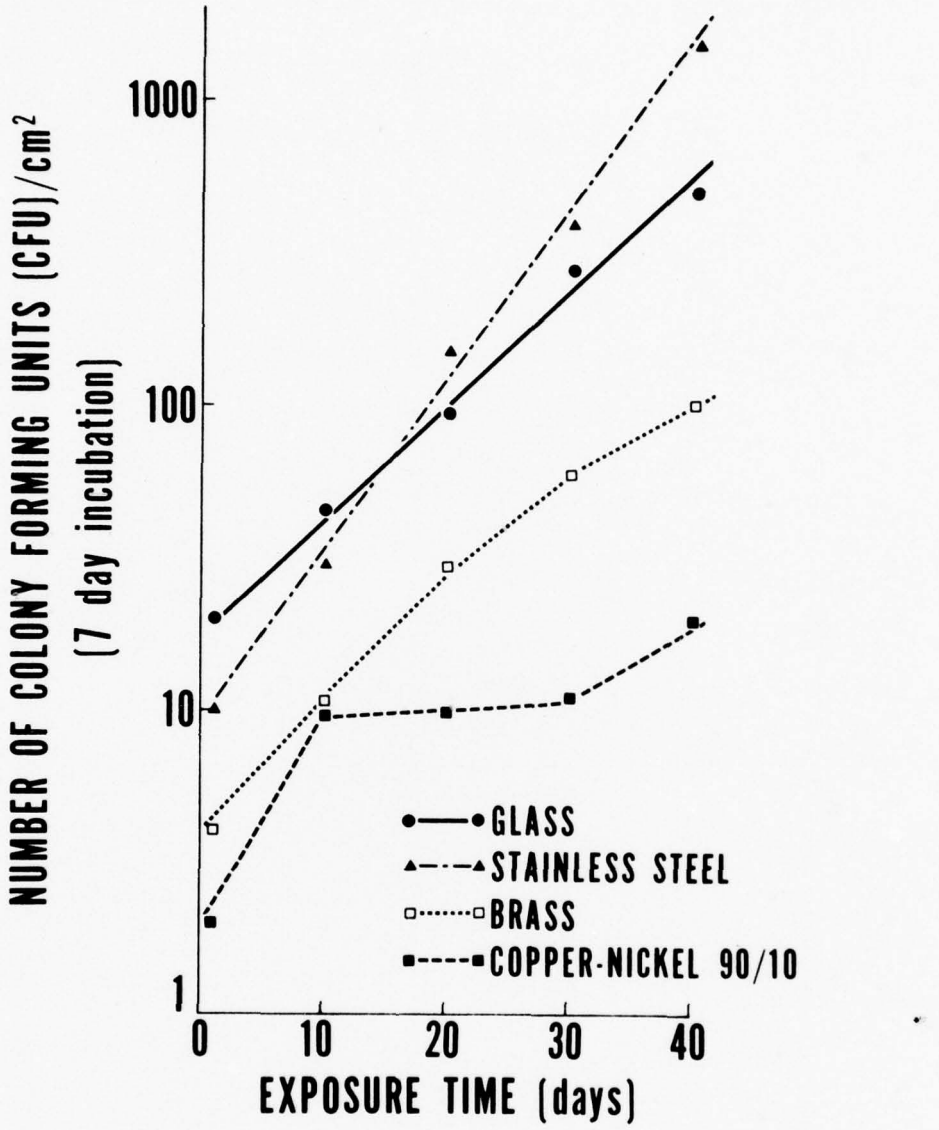


Figure 10. Number of marine heterotrophic bacteria cultured from various substrates in relation to exposure time.

Colonization of brass surfaces by heterotrophs was retarded in early stages and proceeded at a slower rate than that for stainless steel. After 40 days, colony counts for brass were only about 100 CFU/cm^2. Copper-nickel 90/10 alloy was even more retardant than brass which resulted in 40-day counts of 20 CFU/cm^2. These counts are only one-fourth that of brass and one-eightieth that of stainless steel.

One major advantage of isolating the bacteria from the surfaces is that they can be further characterized. Characterization of greater than 100 isolates per substrate from glass, stainless steel and brass has shown that not only is there a large divergence in the degree of attachment and colonization, but also in the type of bacteria present. Only about 10% of the isolates from glass and stainless steel were Gram-positive while 30% of those from brass were Gram-positive (Table 1).

Further classification of the Gram-negative populations from these three surfaces based on their oxidation and fermentation of glucose, their growth on McConkey agar and on their oxidase reaction showed a marked difference in the nature of the bacteria from the different surfaces (Table 2). Although this is a very simplified grouping scheme, it clearly shows that populational composition is strongly influenced by the nature of the surface which is being colonized. On glass, 87% of the isolates fall into the first grouping which includes genera such as Vibrio and Aeromonas, while only 24% of brass isolates are of this type. This is likely due to the nature of the brass (or any copper alloy) since members of this group, e.g., Vibrio alginolyticus, have been shown to be lethally affected by relatively low levels of copper ions (Cabelli, personal communication). The percentage of isolates from stainless steel (60%) falling into this group was intermediate to those from the other groups.

Table 1: Gram stain characteristics of heterotrophic bacterial isolates from the surfaces of glass, stainless steel (304), and brass (60/40 Cu/Zn) held in Miami, Florida seawater.

Characteristics	No. of Isolates		
	Glass	Stainless Steel	Brass
Total isolates	121	122	104
Gram -	109	109	72
Gram +	11	9	28
Gram variable	1	4	4

Table 2: Percentages of heterotrophic, Gram-negative bacterial isolates separated on the basis of three physiological characteriatics.

Physiological Classifications			Percent of Gram Negative Isolates		
MO/F[a]	MCONK[b]	OXID[c]	Glass	Stainless Steel	Brass
+/+	+	+	87	60	24
+/+	+	-	1	0	3
+/+	-	+	3	12	8
+/-	+	+	2	0	0
+/-	-	+	1	2	4
-/-	+	+	1	10	0
-/-	-	+	2	8	15
+/w	+	+	2	0	0
+/w	-	+	0	1	18
w/w	+	+	2	3	0
w/w	-	+	0	4	22
Others			0	1	6

[a]Glucose Marine Oxidative/Fermentative medium: + = acid, - = no change or basic, w = weak acid (Young and Udey, 1976)

[b]McConkey Agar: + = growth in 7 days, - = no growth

[c]Oxidase reaction: + = positive, - = negative
Using 1% N,N,N',N'-tetramethyl p-phenylenediamine

The ability of the isolates to grow on acetate, aspartate, glucose, glutamate, and glycollate as sole carbon sources was also examined. Essentially the same pattern was observed with each carbon source (Figure 11). In all cases, more cultures from glass grew on sole carbon sources than did those from stainless steel, and even fewer cultures from brass grew on these carbon sources. Glutamate was the carbon source supporting the growth of the largest number of isolates. Aspartate was also readily utilized followed by glucose and acetate.

Glycollate was not readily utilized, at least as a sole carbon source, by any group. As a group, the cultures from brass were more nutritionally fastidious and generally slower growing.

A larger portion of the brass cultures (and those from copper-nickel alloys) produce mucoidal matter than do those from glass or stainless steel. This complicates both enumeration of the bacteria (due to their tendency to adhere to one another) and purification of cultures. It further points out the need for using multiple methods for characterizing and enumerating the organisms in microbial films.

Enumeration of bacterial-like cells by epifluorescence directly on metal surfaces is relatively easy on samples exposed for short time intervals. After formation of inorganic products and/or organic films on the surfaces, definition becomes more difficult. When large amounts of nonbacterial growth (e.g., diatoms, filamentous algae and protozoans) colonize the surfaces, these further obliterate the bacteria. Scraping the surfaces and affixing the scraped-off bacteria onto the surface of counter-stained Nucleopore membranes, followed by staining with acridine orange, has proven to be far superior to direct counting and can now be reliably used to obtain "total bacterial counts." Further studies must be conducted to determine the staining properties of bacteria from films.

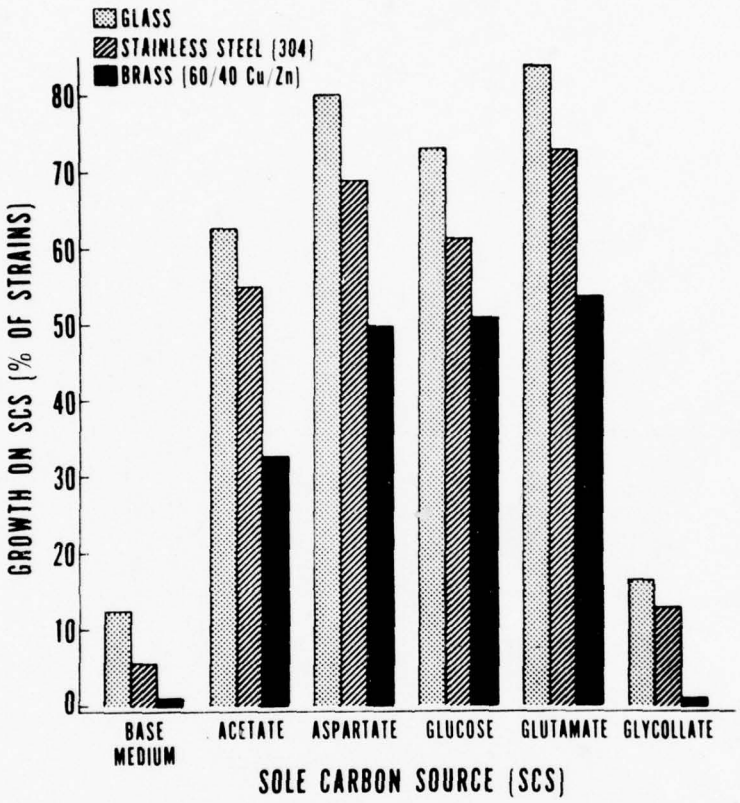


Figure 11. Utilization of sole carbon sources by bacterial isolates.

In the system we described for viewing acridine fluorescence, the majority of bacteria fluoresced in the red-orange spectrum. However, in some instances large masses of green fluorescing bacteria were present. This type fluorescence has been attributed to the fluorescence from dead cells, while other investigators have suggested that different bacterial types fluoresce at different wavelengths (Trolldenier, 1973).

The occurrence of filamentous fungi and of yeasts on surfaces of stainless steel and glass substances and in Biscayne Bay, Florida seawater was also reported earlier (Gerchakov et al., 1976). Briefly, it was found that although all the genera isolated from the substrate surfaces were also found in the water, differences were seen between the metal and glass surface populations. While Cephalosporium, Alternaria and Dactylaria genera were present only on the metal, Torula, Pestalotia and Monotospora were found exclusively on the glass surfaces. It was also noted that yeasts, especially the monomorphic forms such as Cryptococcus spp. were isolated on occasion from the seawater but were conspicuously absent from the two surfaces investigated.

Subsequently, a time study of the fungal population of the tank seawater revealed that although the mycologic taxa that were present varied from sample to sample, no discernible pattern of changes in the population-structure were observed. However, Aureobasidium pullulans, a cosmopolitan taxon in subtropical marine environments (Roth et al., 1964), was always present. This organism occurred in erratic bursts of high numbers and frequently dominated the mycoflora in the water samples.

Additional information was obtained in two additional studies of periphytic fungi where different metal, as well as glass substrates, were examined. Although fungi were cultured from glass slides after only 48 hours of

exposure, persistent residence on the glass by filamentous Deuteromycetes did not occur until the sixth day of immersion. Similarly, persistent surface inhabitation of the stainless steel surface by fungi was also established by the sixth day. In the case of the copper-nickel and brass coupons, fungi could not be cultured until the sixteenth day of immersion. In contrast to the brass and copper-nickel surfaces, the stainless steel supported a larger and more diverse resident fungal population from the third day on. As previously observed, one or more of the yeast-like fungi in the genera Candida, Rhodotorula, and Cryptococcus were routinely isolated from the tank water, often in relatively high populations. Representatives of one or more of these taxa were isolated on only three occasions from stainless steel coupons and twice from glass. On no occasion, however, were these growth forms cultured from the brass or the two copper-nickel alloys immersed in seawater for up to 34 to 92 days, respectively. The observation that yeasts do not readily colonize surfaces may be explained, at least in part, by the rather strict unicellular nature of certain of these yeasts which makes their attachment to a substrate more difficult than for those fungi which have a multicellular structure composed of branching filamentous elements. On the other hand, filamentous fungi were cultured from the copper-nickel coupons and glass slides until the end of the immersion period. In the case of the brass and stainless steel, however, cultures from the surfaces of each metal failed to yield fungi beyond 26 days of immersion in the seawater.

The ability of a fungus to establish residence on a surface may be enhanced if it has the capacity to quickly produce filamentous elements with root-like or "hold-fast" capacity. The myceliated fungi would qualify in this regard as their spores upon germination produce hyphae which contact a surface

and provide anchor. Synthesis of polysaccharidial "glues" and organic acids capable of eroding or etching a metal will further enhance the ability of an organism to adhere to surfaces. The metal-resident fungi A. pullulans and Rhodotorula rubra, produce copious amounts of dextran-like extracellular products which endow the cells with a viscous character. Similarly, the most common filamentous Deuteromycetes growing on the surface of the metal coupons, species of the genus Cladosporium, proved to be the most active and vigorous producers of organic acids. Interestingly, Cladosporium spp. particularly C. resinae have been implicated as a major cause of microbial corrosion of kerosene-type fuel storage tanks and fuel tanks of aircraft (e.g., Hendey, 1964).

The four species of the genus Aspergillus and the three of the genus Penicillium frequently isolated from the coupons, were also found to be capable of rapid and significant acid production.

In summary, it was found that for the substrates under investigation, bacteria were the first colonizers of surfaces immersed in seawater. Fungi appeared later with the lag time dependent on the substrate under consideration. It appeared in all cases that population-structure and physiological characteristics of the resident periphytes were affected by the nature of the substrate.

CORROSION

Because corrosion and corrosion products may affect microfouling and vice versa, weight-loss measurements may aid in interpreting the microfouling behavior which we have observed. Weight loss of stainless steel 304 as a function

of time is described in Figure 12. The rate of weight loss increased by an order of magnitude after about 35 days exposure. This changeover coincided in time with the appearance of the second-tier microfouling layer on the same specimens (Gerchakov et al., 1976). Larger standard error of the mean for data obtained after the changeover was due to the randomized heavy pitting observed on replicate stainless steel specimens. In contrast, as seen in Figure 13, the brass substrate exhibited a different corrosion behavior. The overall corrosion rate was about an order of magnitude smaller than that for the stainless steel, there was no changeover in the rate of weight loss, and the standard error of the mean values were smaller than for stainless steel. This is consistent with the absence of a changeover in the biofouling sequence on the brass substrate, and with the absence of observable pits.

The corrosion rate observed for 90/10 Cu/Ni (Figure 14) was generally higher than that for the 70/30 alloy (Figure 15). The standard error of the mean values for the 90/10 alloy were larger than those for 70/30 and were the result of a higher incidence of randomized pitting.

For comparison, the corrosion rates for the various substrates are arranged in the following descending order: stainless steel, brass, 90/10 Cu/Ni, and 70/30 Cu/Ni. Interestingly, the number of heterotrophic bacteria on the surfaces followed a similar order except that the 70/30 harbored somewhat larger numbers of periphytes than the 90/10 alloy. Until more data are available, however, no conclusion is warranted as to the cause and effect relationship in the microfouling/corrosion phenomenon.

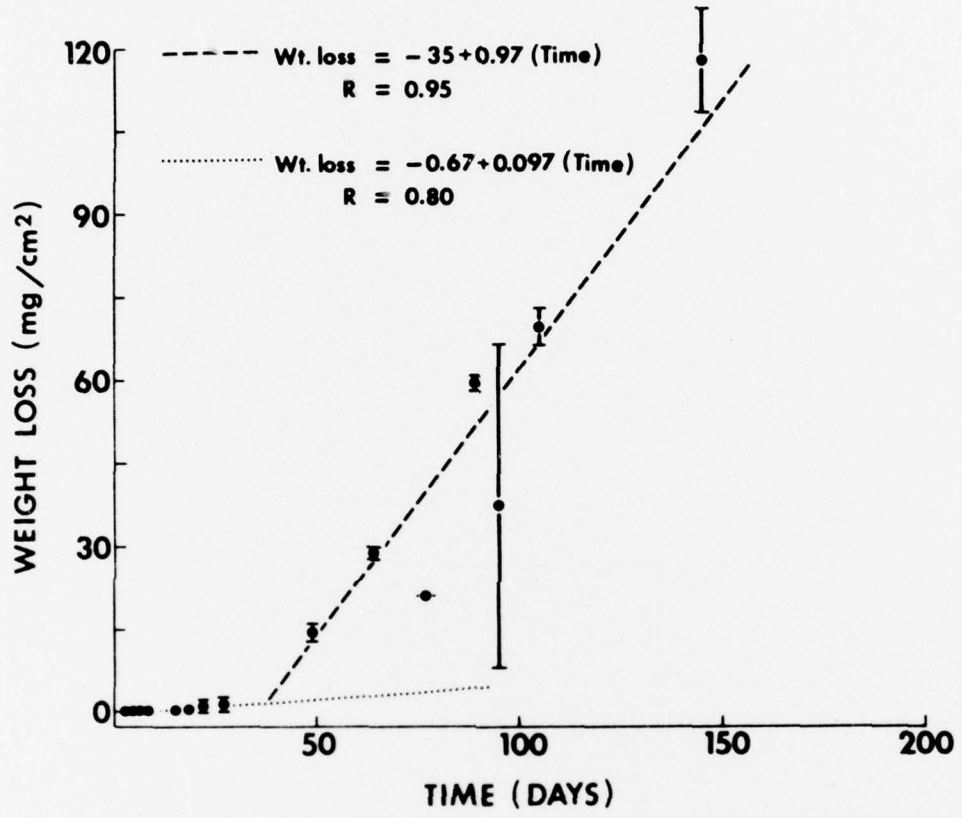


Figure 12. Weight loss as a function of time for stainless steel 304 substrate in Biscayne Bay, Florida seawater.

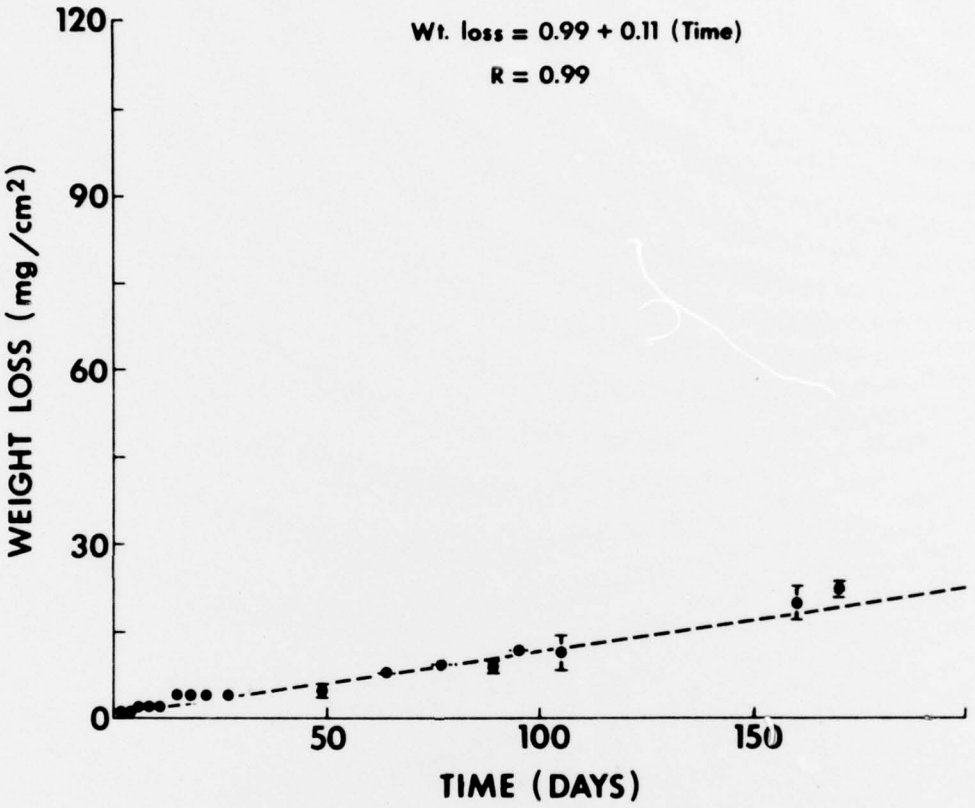


Figure 13. Weight loss as a function of time for brass Cu/Zn 60/40 substrate in Biscayne Bay, Florida seawater.

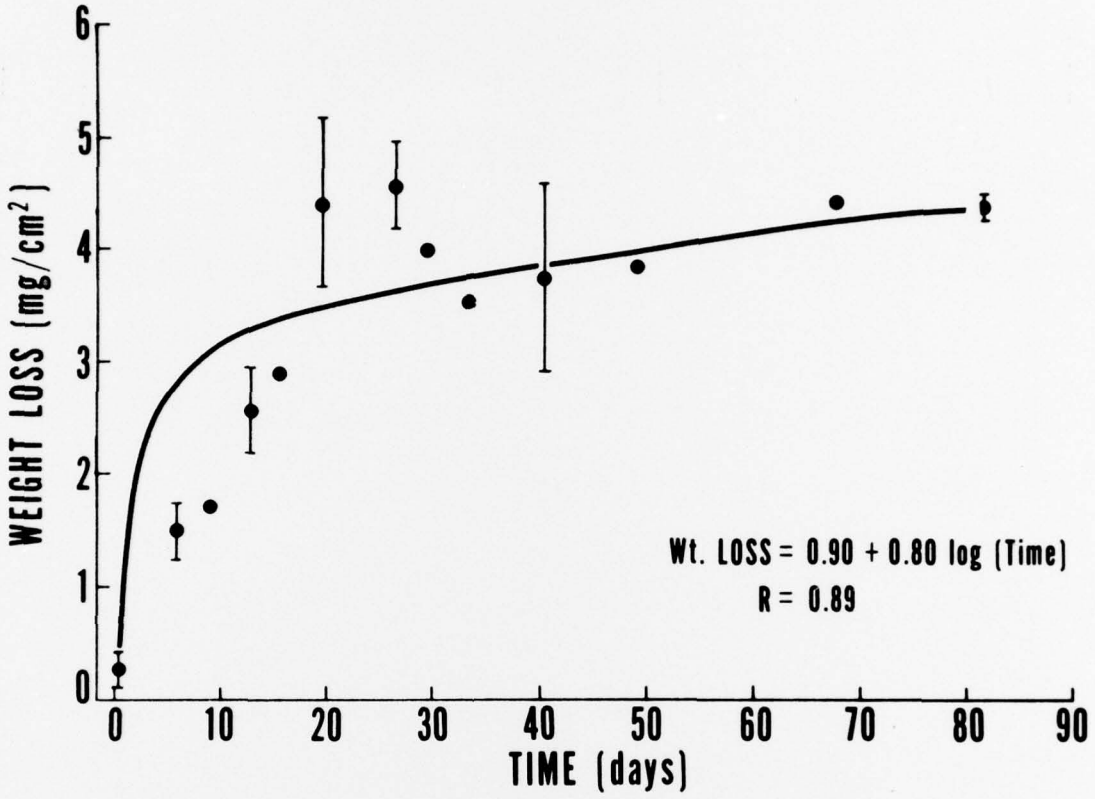


Figure 14. Weight loss as a function of time for 90/10 copper-nickel substrate in Biscayne Bay, Florida seawater.

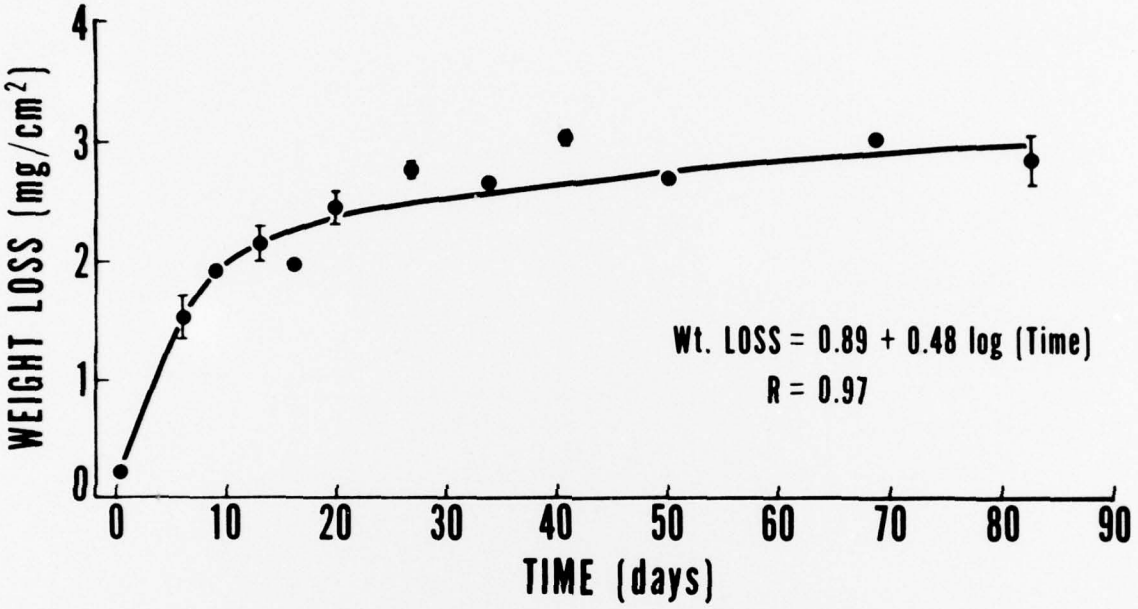


Figure 15. Weight loss as a function of time for 70/30 copper-nickel substrate in Biscayne Bay, Florida seawater.

IMPLICATIONS AND RECOMMENDATIONS

The fact that a primary fouling layer starts to develop on solid surfaces immediately upon their exposure to natural seawater is indisputable. Although a microfouling layer on the surface of a heat exchanger tube is recognized as a potentially detrimental factor in the operation of an OTEC facility, many questions arise as to the magnitude of the expected problems and the proper preventative/curative measures to be taken.

It is apparent that in the absence of fundamental information on the nature of the microfouling/corrosion phenomenon, sound relevant engineering decisions cannot be made. For example, what is the best alloy to be used in the construction of heat exchanger tubes; should there be a laminar or turbulent water flow in the heat exchanger tubes, and at what velocity; what procedure should be employed in removal of the fouling layer; where should an OTEC plant be located.

We may conveniently classify the ELEMENTS involved in microfouling and corrosion of heat exchange surfaces into those which are living and those which are not. The non-living ones can be separated into a solid phase (metal), and a solution phase (seawater). The relative contribution of each of these ELEMENTS to the problem at hand is a function of their intrinsic properties, i.e., population composition of the biota, chemical composition of the metal and of seawater, temperature, etc. The ELEMENTS interact with one another as schematically shown in Figure 16.

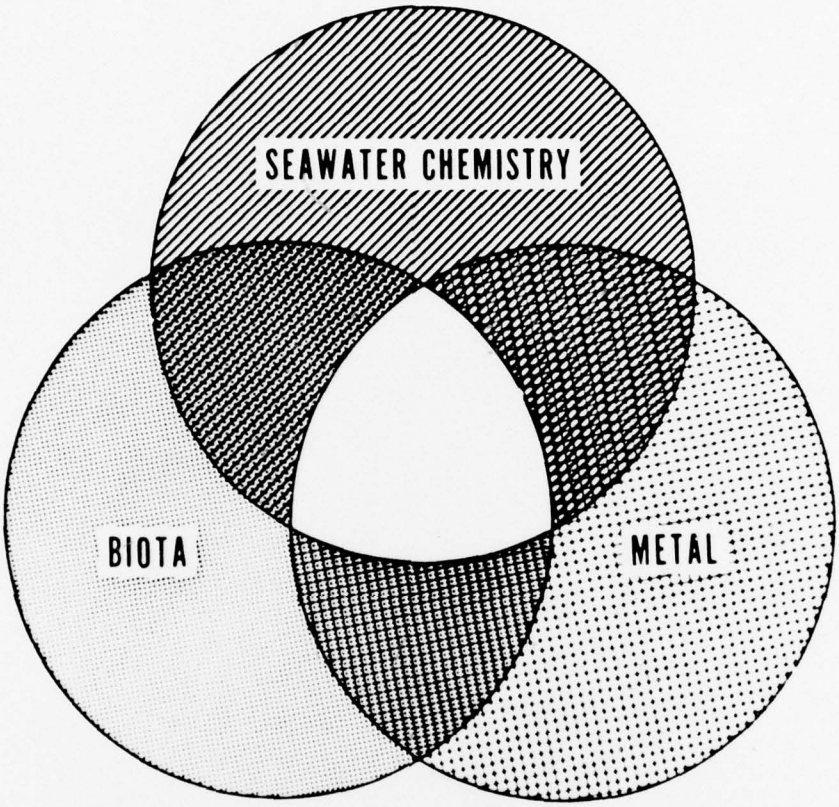


Figure 16. Interaction of the three ELEMENTS which contribute to the microfouling/corrosion phenomenon.

As an illustration we may consider the following hypothetical case. An assemblage of planktonic microorganisms, whose composition is undoubtedly influenced by the intrinsic chemical properties of the ambient seawater, and whose metabolic activities contribute to the chemistry of the seawater, comes in contact with a metal surface. The eventual character of the periphytic community will be determined not only by the characteristics of the individual members, but also by the physico-chemical regime at the metal/water interface. This regime is influenced not only by the properties of the metal substrate and of the seawater, but also by the nature of the interaction between these two ELEMENTS and by the FACTORS (i.e., temperature, hydrodynamics of flow, etc.) controlling this interaction. In turn, the presence of a periphytic community on the surface will have an effect on the metal/water interaction. It follows that the microfouling/corrosion phenomenon is a composite of all the elements as shown at the center of Figure 16.

Realistically, the best one can hope for is to minimize the problem by manipulating the FACTORS which control the interactive zones between any two ELEMENTS and most importantly, manipulating the FACTORS which control the interactive zone among all three ELEMENTS. Information is desperately needed regarding any and all the FACTORS involved. Furthermore, in order to institute effective curative measures, information must be attained on the nature of the inevitable microfouling layer and its adhesion properties.

The data presented in this paper clearly indicates that the nature of the substrate influences the rate of formation, the geometry, and the composition of the microfouling layer. In addition, differences in the physiological

characteristics of the bacterial isolates were also seen, e.g., bacteria residing on copper based alloys were noted for the profuse production of acidic polysaccharide material. We do not know whether there are differences in the heat transfer properties among the various kinds of fouling layers, nor do we know whether one type of layer is more corrosive than another.

During the course of investigating the biofouling/corrosion phenomenon we have employed numerous techniques including transmitted and epiluminescence light microscopy, fluorescence light microscopy, standard microbiological culture techniques, scanning electron microscopy, and energy dispersive X-ray microanalysis. Each yielded specific types of information not readily available by other means. At times, some of the techniques were overlapping in terms of the expected data. We found that a concurrent use of several methodologies served well not only to cross-check the data but to gain information otherwise unobtainable through any single technique. Furthermore, it was possible to modify a given technique, when warranted, without substantially losing valuable information.

Scanning electron microscopy proved to be one of the most useful means of characterizing biofouling films and corrosion products. Not only did the SEM provide a unique type of information, but equally as important, it enhanced information acquired by other methodologies. Also, we routinely used scanning electron microscopy to evaluate the efficiency of other investigative techniques. Microbiological plating techniques, for example, indicated that heterotrophic bacteria are not present on copper based alloys exposed to seawater for less than a few days. Scanning electron microscopy of those coupons however, revealed that bacteria (probably those that would not grow on MMA plates) were present

within a few hours of exposure. They were usually accompanied by abundant flagellates and in some cases fungi and particulate detritus. SEM also revealed the presence or absence of an oxide layer and other information leading to a more complete characterization of the fouling layer. Unfortunately, the SEM can not be used to identify or even enumerate specific types of bacteria, fungi, blue green algae or many types of green algae.

The scanning electron micrographs included in the report (Figures 1-7; also in Gerchakov et al., 1976) illustrate the broad type of information obtainable by this technique. Spatial relationships among cells, and between the biofouling layer and inorganic components, can be evaluated, and some groups of organisms can be identified and enumerated.

In view of our experience in characterizing microfouling layers, and in view of the potential importance of fouling layer studies to the ultimate success of an OTEC system, we recommend that not only multiple methodologies be used concurrently, but that scanning electron microscopy be one of the investigative methodologies. Scanning electron microscopy is the only investigative technique which allows the direct examination of intact fouling layers at high resolution. Caution must be exercised in interpretation of the data, however, because of the possibility of introducing artifacts during specimen handling and preparation, and because of the non-optical nature of the information (for example, the appearance of any surface viewed in the SEM is dependent on the accelerating voltage of the electron beam, the energies of the image forming electrons, specimen conductivity, the procedure used for preparing biological specimens, and other factors). Because of the fragile nature of the biofouling layer, specimen handling becomes an important factor in insuring that data derived by

the various methodologies (including SEM) accurately reflects the nature of the microfouling layer in the OTEC heat exchange tubes. In recognizing these limitations, however, specimen handling procedures can be devised which eliminate or greatly reduce the possibility of altering the characteristics of the fouling layer before it is subject to examination. The basic problem of removing a small section of heat exchange pipe with minimal disruption of the biofouling layer, for example, has been ingeniously solved by Ms. Brenda Little, microbiologist with NORDA (personal communication). Ms. Little's technique involves the prescoring of grooves on the outer-pipe surfaces outlining the specimen sample without disturbing the inner surfaces. Cutting and removing the specimens from the prescored pipe after exposure, can be accomplished easily without introducing artifacts due to cutting vibrations.

The development of a "microfouling/corrosion index" is recommended. This index should be a function of the contributing ELEMENTS and as many controlling FACTORS as realistically possible. In the context of OTEC program objectives, the INDEX should be derived for the various base-alloys under consideration for heat exchanger tubes, and account for FACTORS such as environmental conditions (temperature, seawater chemistry, etc.), and hydrodynamics of flow through the tubes. Although standardized methodologies should be employed to provide a basis of comparison, flexibility and a degree of overlap must be maintained so that techniques can be modified as the need arises.

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Bacterial films	Stainless Steel
Primary films	Brass
Corrosion	Copper Nickel

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Solid surfaces exposed to seawater become populated with bacteria in as little as four hours. Subsequent colonization by a variety of microorganisms produces a complex microfouling layer including their extracellular metabolites and cellular breakdown products, water-borne detrital material, and metal corrosion products (on metal surfaces). The presence of such a primary film on a heat exchange surface may well hinder heat transfer and may be critical to an OTEC system already operating at a low theoretical Carnot efficiency. Furthermore, the metabolic activity within this microcosm may

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enhance corrosion processes.

The succession of periphytic microorganisms was observed for a variety of surfaces, including glass, stainless steel, brass and copper-nickel alloys, submerged in natural seawater. The nature of the periphytic community was influenced more by the composition of the substratum than by the nature of the background planktonic microbiota.